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STUDIES ON THE EFFICACY OF *ALOE VERA* ON ANTIMICROBIAL ACTIVITY

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ABSTRACT

The aqueous, ethanolic, methanolic, petroleum ether and acetone extracts of *Aloe vera were* screened for anti-microbial activity using the Minimal Inhibitory Concentration (MIC) method. They were tested against five bacteria (*Escherischia coli, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumonia* and *Staphylococcus aureus*). The susceptibility of the microorganisms to the extracts in different concentrations plants were compared. The active component in the extract was determined using HPLC technique. The main aim of this study was to find anti-microbial activity of A*loe vera* and to find the presence of active compounds present for the treatment of disease caused by pathogenic microorganism .

KEYWORDS: anti-microbial activity, pathogenic microorganism, HPLC, A*loe vera*

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INTRODUCTION

The use of medicinal plants as a source for relief from illness can be traced back over five million years. Neanderthal who lived 60,000 years ago used many plants which are still widely used in ethano medicine. Potential of many plants as a source for new drugs is still unexplored. Only a small percentage has been investigated for phytochemical, pharmacological or biological function[1].

Plants remain the most common source of antimicrobial agents. Their usage as traditional health remedies is most popular for 80% of world population in Asia and all over the world and is reported to have minimal side effects[2].

In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population. The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources. Because of its wide usage and availability, this study was set out to investigate the antimicrobial activity of the *Aloe vera* .

Aloe vera Linne or *Aloe barbadensis* Miller is a succulent from the Aloe family (400 different species) with its origin in African continent. Its thick leaves supply water for the plant to survive long periods of drought[3]. *Aloe vera* juice is considered helpful for relieving many types of gastrointestinal irritation and juice products are widely available. The concentrated extracts of dried *Aloe leaves* are used as laxative and in hemorrhoid treatment. *Aloe* gel is the most widely recognized herbal remedy in the United States today. It is used to relieve thermal burn, sunburn and promote wound healing[3]. In addition, research suggests that *Aloe* vera has antimicrobial and can help stimulate the body's immune system[4]. Hence a study was carried out to evaluate the antimicrobial activities of *Aloe* vera.

MATERIALS AND METHODS

Plant Material Collection And Extraction

Fresh leaves of *Aloe vera* were collected from Tiruchirappalli district, Tamilnadu, India in October 2010. The material was identified by Rev. John Britto, The Rapinat Herbarium at St. Joseph's college, Tiruchirappalli, Tamilnadu, India. A voucher specimen (KA001 2011/01) was deposited in the St Joseph's college, Tiruchirappalli, Tamilnadu, India.

The plant was brought to the laboratory and thoroughly washed in running water to remove debris and dust particles and then rinsed in distilled water. The leaves were extracted with different solvents in the order of increasing polarity. The solvents used were aqueous, ethanol, methanol, petroleum ether and acetone.

For the preparation of aqueous extract ten grams of fresh plant leaves were soaked in 100ml in distilled water and homogenized, then filtered through Whattman filter paper no. 1. and used for further experiment. For the

preparation of ethanol, methanol, and petroleum ether extracts, fresh leaf gel was dried in the oven at 80°C for 48 h. and then powdered. Ten grams of this powder was soaked in 100ml of each of the solvents namely ethanol, methanol, and petroleum ether for 24 h. The contents were then filtered through Whattman filter paper no. 1 and the filtrate was evaporated to dryness. This dried extract was further powdered and then dissolved in distilled water. Acetone extract was prepared in a similar manner except that the extracted powder was dissolved in 0.15N NaOH and was further neutralized with 0.15N HCl[5].

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the plant extract was carried out for the extracts with maximum antibacterial activity as per the methods and tests given by[6].

The phytochemical compounds in *Aloe vera* were determined by using High Performance Layer Chromatography (HPLC) Technique (Waters). The HPLC equipment consisted of a Waters 2695 Separations Module System equipped with a Waters 996 PDA, UV detector and Empower Chromatography Manager version 1 software (Waters). Separations were done in the isocratic mode, using acetonitrile:water (40:60; v/v) at a flow rate of 1 mL min-1; with an injection volume of 70 µL; UV detection was at 274 nm. All solvents were HPLC grade (Merck). Authenticated sample aloe-emodin of 95% purity by HPLC was supplied by Sigma Chemical Co.

Collection And Maintenance Of Test Organisms.

Pure cultures of the bacterial isolates *(Escherischia coli, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumonia and Staphylococcus aureus)* were collected from the Department of Microbiology, Shrimati Indira Gandhi College, Tiruchirappalli. The organisms were maintained on agar slants stocks and were subsequently sub cultured into newly prepared nutrient agar slants.

Experimental Design

The aqueous, ethanol, methanol, petroleum ether, acetone extracts of *Aloe vera w*as tested to determine the Minimal Inhibitory Concentration (MIC) for each of the bacterial sample. Five reference bacterial samples (*Escherischia coli, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumonia* and *Staphylococcus aureus*) were grown in 10 ml of nutrient broth separately for 24 hours. The different extracts at different range (100,200,400,600 mg) were tested for minimal inhibitory concentration on each bacterial sample. After incubation for 24 h at 37ºC the MIC of each sample was determined by measuring the optical density in the spectrophotometer (620nm), and blank as non inoculated nutrient broth[7].

RESULT AND DISCUSSION

In the present study, preliminary qualitative phytochemicals screening of aqueous extract of *Aloe vera* was carried out. The screening revealed the presence of phytochemicals like tannins, Phlobatannins, saponnin, Flavonoides, terpenoids, cardiac glycosides.

The ethanolic extract of *Aloe vera* was subjected to High Performance Layer Chromatography (HPLC) Technique. The plate was scanned at 274 nm. The analysis of Rf and bands indicated the presence of a anthraquinones which may be Aloe emodin (Figure I) bioactive phytochemical in the band was reconfirmed by running authenticated sample of aloe emodin.

The ethanol, methanol, acetone, petroleum ether, and aqueous extracts of *aloe vera* were subjected MIC against five standard bacteria (*Escherischia coli, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumonia and Staphylococcus aureus.).* It was clear from Table I that both petroleum ether and aqueous extracts of *aloe vera leaves* showed no activity against all organisms tested, unlike its methanol, ethanol and acetone extracts. No remarkable difference in the concentration was observed among inhibitory effect at different concentration. Methanolic, ethanolic, acetone extract showed antibacterial activity against *E.Coli, Bacillus subtilis*. Acetone extract of *Aloe vera* exhibited pronounced activity against *Pseudomonas aeruginosa* than other methanolic and ethanolic extract. The methanolic extract of *Aloe vera* exhibited pronounced activity against *Staphylococcus aureus* than other acetone and ethanolic extract. This could be due to the phytochemical differences between them in different extract. Ethanol, methanol, and acetone are more polar than that of water. More polar substances would extract more of the compounds embedded within the plant cells. In the study conducted by Martinez *et al.* no antimicrobial activity was reported using, aqueous extract of *A. vera* leaves which was inconsistent with the present study.

Anthraquinone contain phenolic compounds which are found exclusively in the plant sap. The important are aloes, aloin, aloe-emodin and barbaloin, act as a painkillers. They also function as antibacterial and anti virals. It was also reported to have antiseptic properties. They act powerfully as antimicrobials against bacteria, viruses, fungi and yeast.

The presence of pyrocatechol in *Aloe vera*, already reported by Kametani *et al*[8]., further, the antimicrobial action of pyrocatechol was illustrated by Cowan[9]. Pyrocatechol is a hydroxylated phenol, known to be toxic

to micro-organisms. The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to micro-organisms and the increase in hydroxylation which further results in increase in toxicity. Further, phenolics also act by denaturing proteins and disrupting cell membranes. They act as a disinfectant and also effective in presence of organic material and remain active on the surfaces even long after application. The antimicrobial activity of ethanolic, methanolic, and acetone extract may be due to the extraction phenolic compound.

The presence of cinnamic acid in *A. vera* gel is comparable with Duke's Phytochemical Databases[10]. Further, the antibacterial action of cinnamic acid is comparable with the findings of Kouassi and Shelef[11]. They suggested that cinnamic acid inhibits glucose uptake and ATP production in the resting cells of bacteria.

The antibacterial activity of coumaric acid was reported by the studies of Baranowski *et al* 1980[12]. The compound is reported to increase the lag phase of the micro-organisms and is also able to inhibit the enzymatic activity of the micro-organisms[13].

Similarly the presence of ascorbic acid in *A. vera* gel is confirmed with Duke's Phytochemical Databases[10]. The antibacterial activity of ascorbic acid is in agreement with the studies of Vilter and Fite *et al*[14,15]. Ascorbic acid may inhibit micro-organisms by interfering with their cell membranes, enzymatic activity, or genetic mechanisms.

From the present study it was concluded that as global antibiotic resistance by bacteria is becoming an interesting public health concern and the race to discover the new antibacterial agent is on, *Aloe vera* gel along with its compounds with promising antibacterial activity could be used as an alternative herbal remedy.

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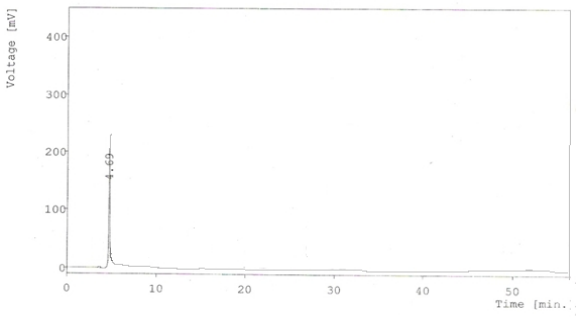
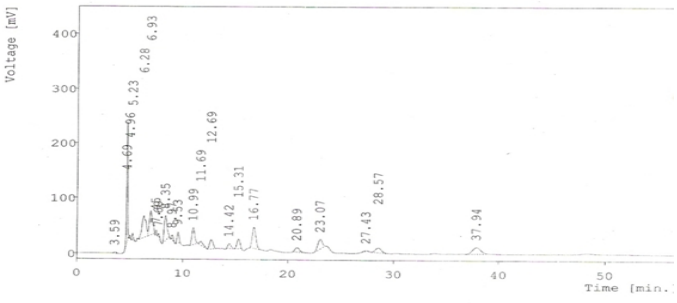


Figure I A HPTC Peak Display Of Authenticated Standard - Aloe Emodin



Result Table

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	3.587	29.1080	1.6382	0.3000	0.3156	0.2894
2	4.687	1490.0724	217.1901	0.1133	16.1551	38.3644
3	4.960	28.2028	5.2348	0.1000	0.3058	0.9247
4	5.233	86.1268	11.3464	0.1333	0.9338	2.0042
5	6.280	1043.9724	38.9199	0.4600	11.3185	6.8748
6	6.933	902.2338	43.1179	0.3467	9.7818	7.6163
7	7.453	50.0584	7.8535	0.1133	0.5427	1.3872
8	7.687	54.4068	6.4415	0.1467	0.5899	1.1378
9	8.347	870.0619	44.0439	0.3267	9.4330	7.7799
10	8.973	96.4460	8.2460	0.1800	1.0456	1.4566
11	9.527	256.8573	19.4010	0.2267	2.7848	3.4270
12	10.993	581.9752	29.9958	0.3067	6.3097	5.2985
13	11.687	174.1812	6.7578	0.3400	1.8884	1.1937
14	12.693	384.9452	16.6408	0.3867	4.1735	2.9394
15	14.420	174.2964	8.2036	0.3400	1.8897	1.4491
16	15.307	341.3827	17.1807	0.3333	3.7012	3.0348
17	16.773	948.3218	38.0902	0.4067	10.2815	6.7282
18	20.887	225.7831	7.7720	0.4733	2.4479	1.3729
19	23.073	490.6650	16.7967	0.4867	5.3197	2.9670
20	27.427	21.6831	0.8514	0.2800	0.2351	0.1504
21	28.567	341.1388	8.6102	0.6467	3.6986	1.5209
22	37.940	631.6450	11.7910	0.8667	6.8481	2.0828
-	Total	9223.5640	566.1234			

Figure I b HPTC Peak Display and its table of *Aloe vera*

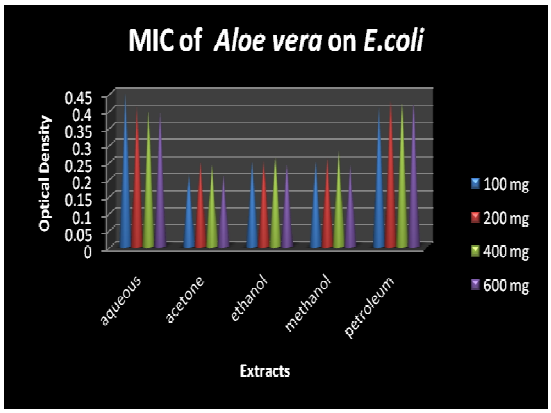


Figure I a MIC of Aloe vera on E.coli

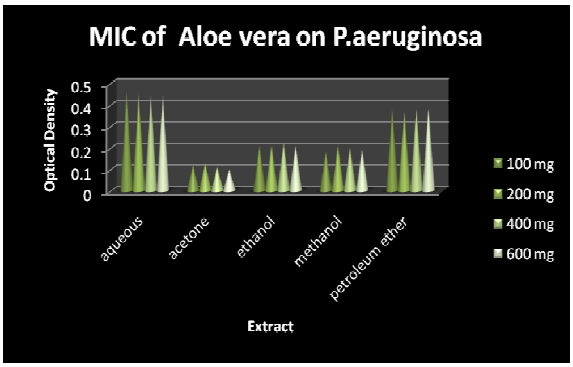


Figure I b MIC of Aloe vera on *Pseudomonas aeroginosa*

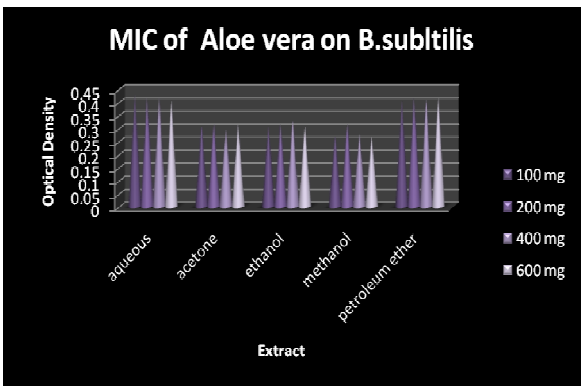


Figure I c MIC of Aloe vera on *Bacillus subtilis*

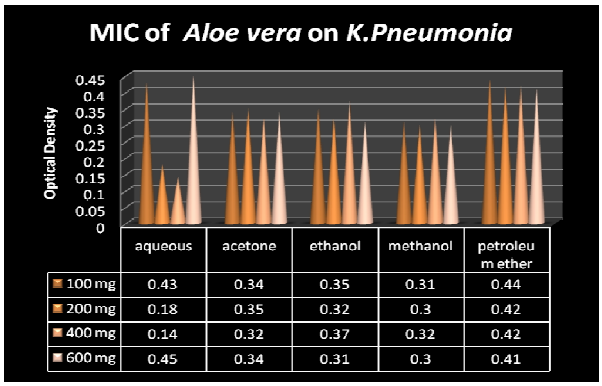


Figure I d MIC of Aloe vera on *Klebsiella pneumonia*

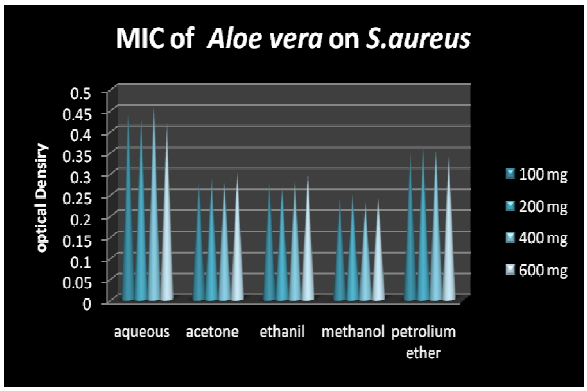


Figure I e MIC of Aloe vera on *Staphylococcus aerues*